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ORIGINAL ARTICLE

Multiple antibiotic resistances and virulence markers of uropathogenic *Escherichia coli* from Mexico

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ABSTRACT

Virulence and antibiotic resistance properties related to different *Escherichia coli* phylogenetic groups have not been studied in detail in Mexico. We aimed to identify patterns of virulence genes and multidrug resistance in phylogenetic groups of uropathogenic strains (UPEC). Strains of *E. coli* were isolated from outpatients with urinary tract infections (UTIs), who went to unit of the public health sector in the State of Mexico. *E. coli* virulence markers and phylogenetic groups were identified by PCR. Susceptibility to 12 antimicrobials was determined by Kirby-Bauer. *E. coli* was identified in 60.4% (n = 194) of the patients with UTIs. Phylogroups B2 51% (n = 99), A 13.4% (n = 26) and B1 10.3% (n = 20) were the most frequent. Resistance to three or up to eleven antibiotics was detected in most phylogroups (n = 188). The genes *fimH* (n = 146), *feoB* (n = 179), *iutA* (n = 178), *sitA* (n = 121), *fyuA* (n = 99), and *traT* (n = 142) were mainly detected in strains of phylogroups B2, A, B1, C, and D. Seventy-two patterns of virulence markers were distributed across eight *E. coli* phylogenetic groups. A high frequency of virulence markers and the multiple antibiotic resistance phenotypes was observed in the phylogroups. The genes of extended-spectrum β-lactamases (ESBLs) found with higher frequency among UPEC strains were bla_{TEM}, bla_{SHV} y bla_{CTX-M} group 1, *CIT* (plasmid-mediated AmpC β-lactamase), and bla_{OXA}-like. In conclusion, our findings show the importance of surveillance, permanent monitoring, and particularly controlled prescription of antibiotics by physicians in the social security health system to reduce the spread of highly virulent UPEC strains that are resistant to multiple antimicrobial agents.

KEYWORDS

Uropathogenic *Escherichia coli*; Virulence factors; Multiple resistance to antibiotics; Phylogenetic groups; Urinary Tract Infections

Introduction

One of the most important bacteria causing urinary tract infections (UTIs) is uropathogenic *Escherichia coli* (UPEC) [1]. UPEC contains numerous virulence genes that encode adhesins, protectins, iron acquisition systems, and toxins, which mediate colonization, invasion, evasion of the immune response, and tissue damage during UTIs [2]. These genes are frequently transferred horizontally between strains by pathogenicity-associated islands (PAIs) [3]. Treatment for UTIs is currently a major challenge due the increase of UPEC strains that are resistant to multiple antibiotics [4]. Phylogenetic analyses have classified *E. coli* into eight major phylogroups; seven belong to *E. coli* sensu stricto (A, B1, B2, C, D, E, and F) and one to Clade I (a cryptic *E. coli* phylogroup) [5]. The associations between virulence factors and the different phylogroups of UPEC strains have been reported [6,7]. In this study we determined the association patterns of virulence genes distributed across the different phylogenetic groups of UPEC strains that are resistant to multiple antimicrobial agents. We also report the frequency of extended-spectrum **β**-lactamases (ESBLs) genes among UPEC strains.

Materials and methods

Bacterial strains

Urine samples were collected from 321 outpatients with signs and symptoms of UTIs (74 men and 247 women, aged between 20 and 70 years) in a family medical unit of the Mexican Social Security Institute, in the State of Mexico. Patients included in the study signed an informed consent agreement and declared that they had not received any previous antibiotic treatment during the last six months. The Ethics Committee of the medical unit approved the study. The collected samples were then subjected to a microbiological analysis. *E. coli* was identified through standard microbiological tests and PCR by amplifying the gene for 16S rRNA [8].

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Susceptibility to antibiotics

Susceptibility to 12 antibiotics (cefotaxime, pefloxacin, carbenicillin, cefalotin, ampicillin, gentamicin, ceftriaxone, netilmicin, chloramphenicol, amikacin, nitrofurantoin, and trimethoprim-sulfamethoxazole) was tested by Kirby-Bauer disc diffusion (Bio-Rad, Mexico). For each test, *E. coli* ATCC 25922 was used as the control. Results were interpreted using the Clinical and Laboratory Standards Institute guidelines [9].

Identification of phylogenetic groups

All eight phylogenetic groups (A, B1, B2, C, D, E, F and cryptic Clade I) were identified using a Quadruplex PCR method following Clermont´s previous description [5].

β-lactamases gene detection

β-lactamases genes were detected by six distinct multiplex-PCR assays according to Dallenne et al. [10].

Detection of virulence genes

Using the PCR method described by Rodríguez-Siek [11], the following genes were detected: adhesion genes, namely, *papA* (pyelonephritis-associated pilus), *papEF* (P-fimbrial adhesin), *fimH* (type-1 fimbriae), *bmaE* (M fimbriae), *focG* (F1C fimbriae), and *gafD* (G fimbriae); iron-acquisition genes *feoB* (ferrous iron cytoplasmic membrane transporter), *iutA* (aerobactin), *ireA* (iron-responsive element), *sitA* (iron transport system), and *fyuA* (yersiniabactin); toxin genes *hlyD* (hemolysin D), protectin/serum resistance; *traT* (Transfer Protein); and *malX*, associated with a pathogenicity island. We defined a pattern of virulence markers as any virulence genes combination distinct from the others.

Table 1. Phylogenetic groups in uropathogenic *Escherichia coli* strains.

Phylogroup	Number (%) N = 194
A	26 (13.4)
B1	20 (10.3)
B2	99 (51)
C	17 (8.7)
D	19 (9.8)
E	0 (0)
F	3 (1.5)
Clade I	1 (0.5)
Nontypeable	9 (4.6)
Total	194 (100)

Results

E. coli was identified in 60.4% (n = 194) of patients with UTIs. Table 1 shows that the most frequently found phylogroups amongst strains were B2 (n = 99), A (n = 26) and B1 (n = 20). Clade I (cryptic) was identified in only one strain. It was not possible to assign the phylogenetic group to nine strains.

The majority of strains in the different genetic groups showed resistance to beta-lactam antibiotics, cefalotin, ampicillin, and carbenicillin (Table 2). Clade I strain showed resistance to 9 of the 12 tested antibiotics, while most strains from phylogroup F showed resistance to 6 antimicrobial agents. Resistance to three and up to 11 antibiotics was detected in most phylogroups (n = 188).

Adhesion genes *fimH* and *papEF* were frequently detected in phylogroups B2, C, and D (Table 3), while markers related to iron acquisition, *feoB, iutA*, and *sitA*, were mostly identified amongst phylogroups B1, B2, and C. Gene *traT* (protectin) was predominant amongst phylogroups A, B2, and C.

Seventy-two different patterns of virulence markers related to *E. coli* phylogenetic groups were observed (Table 4). Pattern number 1 (n = 19), consisting of genes *fimH/feoB/iutA/traT* and pattern number 4 (n = 13), consisting of *fimH/feoB/iutA/sitA/fyuA/traT* were distributed in phylogenetic groups A, B1, B2, C, and D. Pattern number 5, consisting of *fimH/feoB/iutA/sitA/traT* was present in most phylogroups (A, B1, B2, C, D, and F), as well as in two strains without a designated group.

The genes of extended-spectrum β-lactamases (ESBLs) found with higher frequency among UPEC strains were bla_{TEM}, bla_{SHV} y $bla_{CTX\text{-}M}$ group 1, *CIT* (plasmid-mediated AmpC β-lactamase), and bla_{OXA}-like (Table 5).

Table 2. Antibiotic resistance by phylogroup in UPEC strains.

Antibiotic	Phylogenetic group No. (%)						
	A (n = 26)	B1 (n = 20)	B2 (n = 99)	C (n = 17)	D (n = 19)	F (n = 3)	Clade I (n = 1)
Cefotaxime	19 (73)	16 (80)	74 (74.7)	12 (70.6)	12 (63.1)	3 (100)	1 (100)
Pefloxacin	17 (65.3)	18 (90)	79 (79.8)	15 (88.2)	11 (57.9)	3 (100)	1 (100)
Carbenicillin	26 (100)	20 (100)	97 (98)	16 (94.1)	17 (89.4)	3 (100)	1 (100)
Cefalotin	26 (100)	20 (100)	96 (97)	16 (94.1)	18 (94.7)	3 (100)	1 (100)
Ampicillin	26 (100)	20 (100)	96 (97)	17 (100)	17 (89.4)	3 (100)	1 (100)
Gentamycin	8 (30.7)	12 (60)	55 (55.5)	8 (47)	6 (31.7)	1 (33.3)	1 (100)
Ceftriaxone	10 (38.4)	12 (60)	50 (50.5)	11 (64.7)	7 (36.8)	1 (33.3)	1 (100)
Netilmicin	7 (30)	8 (40)	42 (42.4)	8 (47)	4 (21)	0	1 (100)
Chloramphenicol	10 (38.4)	7 (35)	24 (24.2)	4 (23.5)	3 (15.8)	2 (66.6)	0
Amikacin	2 (7.7)	4 (20)	16 (16.1)	1 (5.8)	2 (10.5)	0	0
Nitrofurantoin	12 (46.1)	9 (45)	46 (46.4)	9 (52.9)	9 (47.3)	0	0
Trimethoprim & sulfamethoxazole	16 (61.5)	14 (70)	64 (64.6)	11 (64.7)	14 (73.7)	3 (100)	1 (100)

Table 3. Virulence genotypes in the phylogroups of UPEC strains.

	Virulence markers in UPEC strains (n = 194)													
	Adhesins						Iron-related					Toxin	Protectin	Others
Phylogroup	*papA*	*papEF*	*fimH*	*bmaE*	*focG*	*gafD*	*feoB*	*iutA*	*ireA*	*sitA*	*fyuA*	*hlyD*	*traT*	*malX (PAI)*
A (n = 26)	0	3	17	0	1	0	22	25	6	9	7	1	23	2
B1 (n = 20)	3	3	16	0	0	0	18	17	3	15	12	1	15	0
B2 (n = 99)	26	33	76	0	6	0	94	91	13	68	61	20	65	18
C (n = 17)	4	9	13	0	1	0	17	16	4	13	9	6	16	1
D (n = 19)	4	8	15	0	0	0	16	17	4	9	7	1	14	2
F (n = 3)	0	0	2	0	0	0	3	3	0	1	0	1	2	0
Clade I (n = 1)	1	1	1	0	0	0	0	1	1	0	1	1	1	0
Nontypeable (n = 9)	1	1	6	0	0	0	9	8	0	6	2	2	6	1
No. (%)	39 (20.1)	58 (29.8)	146 (75.2)	0 (0)	8 (4.1)	0 (0)	179 (92.3)	178 (91.7)	31 (24.2)	121 (62.4)	99 (51)	33 (17)	142 (73.2)	24 (12.4)

Table 4. Patterns of virulence genes related with the phylogroups in strains UPEC.

Pattern number	No. of strains (%)	Patterns of virulence genes														Phylogroup (No. of strains) B1 B2 C								
		Adhesins						Iron-related					Toxin	Protectin	V Others									
		papA	*papEF*	*fimH*	*bmaE*	*focG*	*gafD*	*feoB*	*iutA*	*ireA*	*sitA*	*fyuA*	*hlyD*	*traT*	*malX*	A	B1	B2	C	D	E	F	Clade I	Unassignable
1	19 (9.8)			+				+	+					+		4	1	9	2	3	0	0	0	0
2	14 (7.2)			+				+	+		+	+				0	2	10	1	1	0	0	0	0
3	13 (6.7)			+				+	+							3	2	6	0	1	0	0	0	1
4	13 (6.7)			+				+	+		+	+		+		1	4	6	1	1	0	0	0	0
5	13 (6.7)			+				+	+		+			+		3	1	2	2	2	0	1	0	2
6	6 (3.1)			+				+	+		+					1	0	4	0	0	0	0	0	1
7	5 (2.6)		+	+				+	+		+			+		0	0	4	1	0	0	0	0	0
8	5 (2.6)			+				+	+		+	+		+	+	0	0	4	0	1	0	0	0	0
9	5 (2.6)							+	+		+					1	0	2	0	0	0	0	0	2
10	5 (2.6)							+	+					+		3	0	0	1	0	0	0	0	1
11	4 (2.1)			+				+	+		+	+	+			0	0	4	0	0	0	0	0	0
12	4 (2.1)			+				+	+	+	+	+		+	+	0	0	3	1	0	0	0	0	0
13	4 (2.1)			+				+	+		+	+	+	+		0	1	1	1	0	0	0	0	1
14	3 (1.5)			+				+	+				+	+		0	0	1	0	1	0	1	0	0
15	3 (1.5)	+	+	+				+	+	+	+	+		+		0	0	1	1	1	0	0	0	0
16	3 (1.5)	+	+	+				+	+		+	+	+			0	0	2	1	0	0	0	0	0
17	3 (1.5)	+	+	+				+	+		+	+				0	0	3	0	0	0	0	0	0
18	3 (1.5)		+					+	+		+	+		+		0	0	2	1	0	0	0	0	0
19	3 (1.5)							+	+			+				0	1	2	0	0	0	0	0	0
20	3 (1.5)							+	+			+		+		1	1	1	0	0	0	0	0	0
21	3 (1.5)								+			+		+		1	0	1	0	0	0	0	1	0
22	2 (1.0)			+		+		+	+		+	+	+	+		0	0	2	0	0	0	0	0	0
23	2 (1.0)	+	+	+				+	+							0	0	1	0	1	0	0	0	0
24	2 (1.0)	+		+				+	+							0	1	1	0	0	0	0	0	0
25	2 (1.0)			+		+		+	+	+				+		0	0	1	1	0	0	0	0	0
26	2 (1.0)	+	+	+				+	+		+	+		+		0	1	0	1	0	0	0	0	0
27	2 (1.0)								+		+		+	+		0	0	2	0	0	0	0	0	0
28	2 (1.0)	+	+					+	+		+	+				0	1	1	0	0	0	0	0	0
29	2 (1.0)							+	+		+	+		+		1	0	1	0	0	0	0	0	0
30	2 (1.0)		+	+					+					+		1	0	0	0	1	0	0	0	0
31–72	42 (21.6)	Different combinations of genes														6	4	22	2	6	0	1	0	1
Total	194 (100)															26	20	99	17	19	0	3	1	9

Table 5. Distribution of β-lactamase genes in UPEC strains.

Gene		No. of strains (n = 194)	%
bla_{TEM}		43	22.1
bla_{SHV}		27	13.9
bla_{OXA}-like		52	26.8
bla_{CTX-M} group 1		47	24.2
bla_{CTX-M} group 2		0	0
bla_{CTX-M} group 9		0	0
bla_{CTX-M} group 8/25		0	0
plasmid-mediated AmpC	*ACC*	0	0
β-lactamase genes	*FOX*	0	0
	MOX	0	0
	DHA	0	0
	CIT	46	23.7
	EBC	0	0
bla_{VEB}		0	0
bla_{PER}		0	0
bla_{GES}		0	0
bla_{IMP}		0	0
bla_{VIM}		0	0
bla_{KPC}		0	0
bla_{OXA-48}-like		9	4.6

Discussion

The emergence of *E. coli* strains resistant to multiple antibiotics is considered a serious health issue that hampers treatment of UTIs [12]. This difficulty has increased due to the high frequency of virulence genes in UPEC strains, boosting pathogenicity during infections [13]. In this study we have investigated virulence- and antibiotic resistance- markers in 194 *E. coli* strains, belonging to distinct phylogroups, isolated from patients with UTIs. The analysis of our results showed that the UPEC strains were distributed in seven phylogenetic groups (Table 1), with phylogroup B2 being the most prevalent, followed by phylogroups A, B1, and D (Table 1); these data also coincide with those on the phylogroups found recently on uropathogenic strains of *E. coli* [14]. Most strains in these seven phylogroups were resistant to 3–7 antibiotics (data not shown), including beta-lactams carbenicillin, cefalotin, ampicillin, and cefotaxime, as well as pefloxacin and trimethoprim-sulfamethoxazole. Resistance to ampicillin, amoxicillin-clavulanate, tetracycline, nalidixic acid and trimethoprim-sulfamethoxazole in UPEC strains is associated with phylogroups B2, A, D, and B1 [15]. The high percentage of strains producing ESBLs we have found (Table 5) is worrisome and is similar to that reported in others parts of world [16]. We hypothetize that the high resistance to multiple antibiotics in the analysed UPEC strains may be the result of extended therapeutic administration use of antibiotic without medical prescription until 2010 in Mexico. This would have caused the spread of multi-resistant strains in the community with the ability to transfer mobile genetic elements horizontally, such as through plasmids, transposons, and integrons. Further studies are needed to evaluate the impact of antibiotic use without medical prescription or antibiotic susceptibility tests in the increase and genetic composition of antibiotic resistant strains [17].

UPEC carries numerous adhesion factors that facilitate colonization, invasion, and internalization during UTI pathogenesis [18]. In this study, we found that the most frequently found adhesion genes in strains were *fimH* and *papEF* (Table 3), mainly associated with phylogroups B2, B1, C and D, which coincides with previous data for uropathogenic strains of *E. coli* [19]. *papEF* was the most frequently observed gene in strains associated with pyelonephritis, and *fimH*, in strains that cause UTIs [20].

Genes encoding iron-acquisition systems facilitate a great number of cellular activities, such as nucleotide biosynthesis, electron transport, and peroxide reduction, which are essential for *E. coli* survival and reproduction [21]. Iron acquisition genes *feoB, iutA*, and *sitA* were more frequently found amongst phylogroups B2, A, B1 and C (Table 3). Our results are similar to those reported for UPEC strains, where *feoB* was detected in phylogroups B2, D, and A, and *iutA* and *sitA* were detected in group B2 [11]. Furthermore, gene *traT* appeared more frequently in groups B2, A, C, A, and D (Table 3); *hlyD*, in B2 and C; and *malX* (PAI), in B2. Gene *traT*, which encodes an external membrane protein involved in serum resistance, has been found in groups B2, A, and D [11] while gene *malX*, which is located in a PAI and encodes a phosphotransferase system enzyme II that can recognize maltose and glucose [22], has been found in phylogroup B2 [23].

In this study, seventy-two different patterns of *E. coli* virulence genes were found distributed mainly in phylogroup B2, followed by phylogroups A, B1, D, and C (Table 4). Different combinations of genes *papA, papEF, fimH, feoB, iutA, sitA*, and *fyuA* were distributed in pattern numbers 15, 16, 17, and 26, appearing mainly in phylogroups B2, D, C, and B1 while different combinations of genes *fimH, feoB, iutA, sitA, fyuA*, and *traT* were found in pattern numbers 4, 8, 12, 13, and 22, appearing mainly in groups B2, B1, A, C, and D. Findings have shown that most *E. coli* strains causing UTIs belong to phylogenetic group B2 [19]. The association of adhesion genes, iron-acquisition genes, and genes coding for protectins, toxins, and pathogenicity islands found in the different phylogenetic groups of UPEC strains, along with genes responsible for resistance to multiple antibiotics, reveal the ability of strains to cause recurrent, chronic, and acute infections, such as cystitis or pyelonephritis. This is the first study, which has been carried out in Mexico, on association patterns of virulence markers related to phylogenetic groups of the uropathogenic strains of *E. coli* that are resistant to multiple antibiotics and carry extended-spectrum β-lactamases genes. In this context, implementing surveillance and monitoring strategies, improving medical treatments to reduce UTIs caused by UPEC, and preventing the spread of multiple drug resistant strains is essential.

Disclosure statement

No potential conflict of interest was reported by the authors.

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